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MIPR NUMBER 96MM6673

TITLE: Dry Fibrin Glue for the Repair of Severe Liver Injury in the Anesthetized Porcine Model

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REPORT DATE: October 1996

TYPE OF REPORT: Midterm

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE
October 1996

3. REPORT TYPE AND DATES COVERED
Midterm (1 Mar 96 - 30 Sep 96)

4. TITLE AND SUBTITLE
Dry Fibrin Glue for the Repair of Severe Liver Injury in the Anesthetized Porcine Model

5. FUNDING NUMBERS
96MM6673

6. AUTHOR(S)
MAJ John B. Holcomb

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
William Beaumont Army Medical Center
El Paso, Texas 79920-5001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*
Liquid Fibrin Sealants are being used for hemorrhage control. Dry Fibrin Sealant Dressings (DFSD) have the potential for improved hemorrhage control in a field environment. We have created a severe liver injury model, and stopped bleeding with a DFSD in an otherwise fatal injury. Bovine and human thrombin injected intravenously have been compared for hemodynamic effects. Blood loss in a fluid versus no fluid resuscitation liver injury model has been compared. We are now ready to prospectively study DFSD versus gauze packing for the control of hemorrhage from a severe liver injury.

14. SUBJECT TERMS
Fibrin Sealant, Hemorrhage Control, Human Thrombin, Bovine Thrombin, Resuscitation

15. NUMBER OF PAGES
14

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

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John Hobbs

PI - Signature Date

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INTRODUCTION: Dry fibrin sealant dressing (DFSD) has been used successfully with significantly decreased hemorrhage and preserved blood pressure in a ballistic extremity injury model.[1] As this dressing has never been applied to a blunt liver injury, the protocol entitled Dry Fibrin Glue for the Repair of Severe Liver Injury in an Anesthetized Porcine Model was submitted. Before the DFSD could be applied to this type of injury, a realistic and reproducible model needed to be developed. This solid organ injury model allows us to study not only fluid resuscitation but most importantly hemorrhage control. All liver injury models currently in the literature suffer from some or all of the following limitations:

1. Nonrealistic injuries, i.e. sharp excision of portion of lobes,possibly appropriate for study fluid resuscitation but not for effectiveness of hemorrhage control.

2. Nonreproducibility of the injury.

3. Heparin is required to induce and maintain bleeding.

4. Requirement of a controlled pre-bleed for standardization of hemodynamic insult.

Over the past 9 months the grant of $25,000.00 (plus the additional $25,000.00 received) has allowed our lab to develop and mature a blunt liver injury trauma model that reproducibly causes a grade V liver injury (injury to one or more of the retrohepatic veins).[2] This type of injury has a 60-90% mortality in humans.[3] Dr. Harris has modified the AAST organ injury system to accurately delineate the injury we are creating (Appendix 1). With the recent upgrade of our lab and the American Red Cross's development of the dry fibrin sealant dressing, we will soon start actual study of the dry fibrin sealant dressing versus gauze packing for control of severe liver injury.

The American Red Cross has encountered minor difficulties in the development of the dry fibrin sealant dressing, impeding our ability to complete the study as outlined. While waiting on the development of the dry fibrin sealant dressing, we accomplished several other important milestones in this project. One, development of the liver injury model with complete characterization of the injury, and confirmation of reproducibility. Critical to the injury and the success of the model is the specific anatomy of the pig liver and hepatic veins, which is very similar to human anatomy. The injury that we create is not only a parenchymal injury (4cm deep) but typically 1-3 holes in the major hepatic veins, which on dissection are up to 13 mm in

diameter and within 2-4 cm of the inferior vena cava. Development and characterization of the injury has proceeded throughout the other studies that I will outline below. At this point we can reliably reproduce a grade V_2 through V_6 injury, which allows us to injure the liver, treat the animal prospectively and then grade the injury retrospectively. This retrospective grading of a prospectively treated injury allows us to correlate the specific injury with the resulting hemodynamic changes.

Placement of gauze packs above and below the liver is the standard method now for control of major liver injuries and humans.[3] There is no data on this treatment of liver injuries in pigs, therefore, this had to be developed, and is now accomplished without difficulty. This is the standard of care portion of the protocol. Important not only to this project using dry fibrin sealant dressing, but to all fibrin sealant hemorrhage control type studies, is the article by Dr. Moore's group out of Colorado, delineating the fatal effects of fibrin glue in deep liver injuries.[4] Bovine thrombin, which is in all fibrin glues currently used in the United States was postulated to cause hypotension and death. This study had two human subjects and a single animal. We studied the hemodynamic aspects of bovine thrombin (BT) versus human thrombin (HT) injected intravenously (8 swine). It is apparent from Appendices 2

and 3 that BT is fatal and HT is not (dose = 5000 IU). Analysis of BT and HT by HPLC reveals that bovine is very impure in comparison to human thrombin. Autopsy data showed that BT swine had extensive intravascular clotting, while HT animals exhibited only normal post mortum changes. We feel these differences may not be due to thrombin but to protein impurities in the BT preperation. This is critical to the acceptance of dry fibrin sealant dressing to the trauma community because of the warnings published by Dr. Moore's group in 1991. This information is currently being collated by Dr. Pusateri for publication.

In addition, we studied in 15 animals the effects of standard fluid resuscitation versus no fluid resuscitation in a grade 4 or 5 liver injury. Results show a significant decrease for the nonrecusitated animals, (2997±660ml vs 468±706ml, p=.02) in blood loss over 1 hour. This data was collected while we were standardizing our liver injury, and will support a separate paper on our liver injury model, currently being written by Dr. Harris. This data also confirms the utility of this model as a realistic type injury (90% fatal in humans) and will allow us to study the type, timing, and amount of resuscitation that maximizes survival. The preceding two studies are preliminary and were pursued during the development of a reproducible liver model.

The American Red Cross, as mentioned previously, has encountered difficulties in the manufacturing of dry fibrin sealant dressing, which are essentially overcome at this point. We have placed prototype dressings into grade V_{2-6} liver injuries and stopped bleeding in 15-30 seconds (four animals). In animals previously studied with fluid resuscitation, this injury has uniformly been fatal. Blood loss in the injured and fluid resuscitated animals averages 2997±660ml. In the four animals treated with the DFSD after injury and with fluid resuscitation, average blood loss is 602±82ml. Clinically patients with these severe liver injuries have gauze packing applied, and the patient returns to the OR in 24-72 hours for gauze pack removal. This return trip to the OR could be avoided if the dry fibrin sealant dressing performs as expected, and replaces gauze packs as the treatment for severe liver injuries.

At this point we are only awaiting the final dressing from the American Red Cross, which should be available to us within 2 months. We will then commence the prospective randomized study of dry fibrin sealant dressing versus conventional gauze packing with fluid resuscitation in our severe liver injury model. This research is important not only because the model almost exactly replicates a type of injury seen in blunt liver trauma, but also evaluates a

unique off-the-shelf, temperature independent, fibrin based dressing that stops hemorrhage almost immediately.

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MODIFIED LIVER ORGAN INJURY SCALE for SWINE

GRADE IV
PARENCHYMA ONLY

GRADE V_1
ONE MAJOR BLOOD VESSEL, NOT THROUGH & THROUGH

GRADE V_2
ONE MAJOR BLOOD VESSEL, THROUGH AND THROUGH

GRADE V_3
TWO MAJOR BLOOD VESSELS, NOT THROUGH AND THROUGH

GRADE V_4
TWO MAJOR BLOOD VESSELS, THROUGH AND THROUGH

GRADE V_5
THREE MAJOR BLOOD VESSELS, NOT THROUGH AND THROUGH

GRADE V_6
THREE MAJOR BLOOD VESSELS, THROUGH AND THROUGH

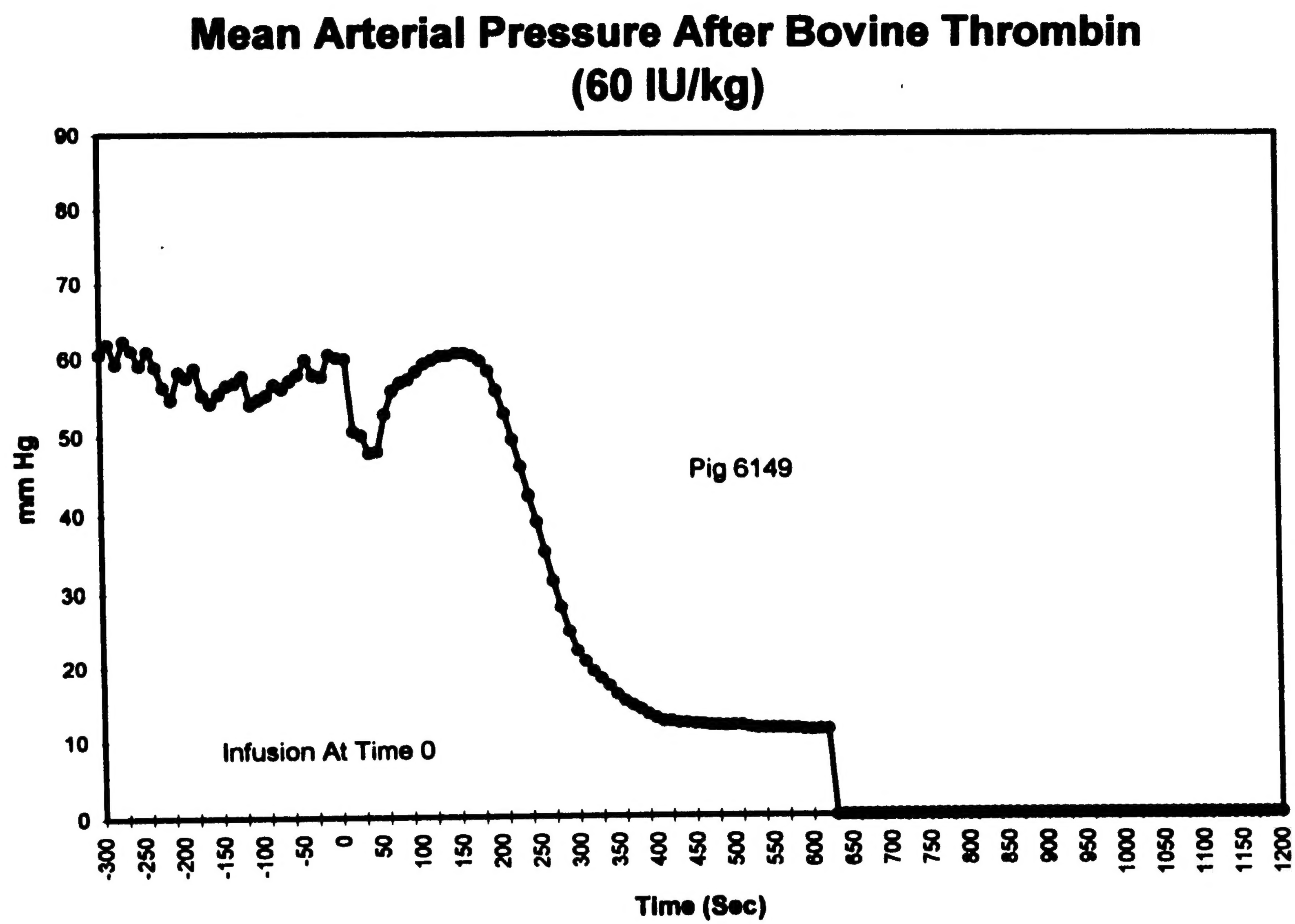
GRADE V_7
VENA CAVA INJURY, NOT THROUGH AND THROUGH

GRADE V_8
VENA CAVA INJURY, THROUGH AND THROUGH

NOTE: MAJOR VEINS, 7-8mm DIAMETER, within 2.0 - 3.0cm of the VENA CAVA

Appendix 2

Chart1



Appendix 3

Chart1

Mean Arterial Pressure After Human Thrombin (66 IU/kg)